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Liver Fat is Associated With Markers of Inflammation and Oxidative Stress in Analysis of Data From the Framingham Heart Study

Zachary P. Fricker, MD[1], **Alison Pedley, PhD**[2], **Joseph M. Massaro, PhD**[3], **Ramachandran S. Vasan, MD**[2,4,5,7], **Udo Hoffmann, MD**[6], **Emelia J. Benjamin, MD**[2,5,7], and **Michelle T. Long, MD**[1,2]

[1]Section of Gastroenterology, Evans Department of Medicine, Boston University School of Medicine, Boston, MA

[2]National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA

[3]Department of Mathematics and Statistics, Boston University School of Public Health, Boston, MA

[4]Section of Preventive Medicine and Epidemiology, Evans Department of Medicine, Boston, University School of Medicine, Boston, MA

[5]Whitaker Cardiovascular Institute and Cardiology Section, Evans Department of Medicine, Boston University School of Medicine, Boston, MA

[6]Radiology Department, Massachusetts General Hospital, Harvard Medical School, Boston, MA

[7]Department of Epidemiology, Boston University School of Public Health, Boston, MA

Abstract

Background & Aims: Non-alcoholic fatty liver disease is an inflammatory condition that results in progressive liver disease. It is unknown if individuals with hepatic steatosis, but not known to have liver disease, have higher serum concentrations of markers of systemic inflammation and oxidative stress

Correspondence: Michelle T. Long, MD, Boston University School of Medicine, Section of Gastroenterology, 85 East Concord Street 7th Floor, Boston MA 02118, mtlong@bu.edu, Tel: (617) 638-8392, Fax: (617) 638-6529.

Author Contributions:

Study concept and design (EJB, MTL); acquisition of data (RSV, UH, EJB); analysis and interpretation of data (ZPF, RSV, UH, EJB, MTL); drafting of the manuscript (ZPF); critical revision of the manuscript for important intellectual content (AP, JMM, RSV, UH, EJB, MTL); statistical analysis (AP, JMM); obtained funding (RSV, EJB, MTL); administrative, technical, or material support (UH); study supervision (MTL).

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Methods: We collected data from 2482 participants from the Framingham Heart Study (mean age, 51±11 years; 51% women) who underwent computed tomography and measurement of 14 serum markers of systemic inflammation. Heavy alcohol users were excluded. Liver:Phantom ratio (LPR, a continuous parameter of liver attenuation relative to a calibration phantom) was used to identify individuals with radiographic evidence of liver fat. Primary covariates included age, sex, smoking, alcohol, aspirin use, hypertension, dyslipidemia, diabetes, and cardiovascular disease. Body mass index and visceral fat were secondary covariates. We used multivariable linear regression models to assess the association between liver fat and systemic inflammatory markers.

Results: In multivariable-adjusted models, liver fat was associated with the following inflammatory markers: high-sensitivity C-reactive protein ($P<.001$), urinary isoprostanes ($P<.001$), interleukin 6 ($P<.001$), intercellular adhesion molecule 1 ($P<.001$), and P-selectin ($P=.002$). Additional adjustment for body mass index or visceral fat attenuated the results slightly, although all associations remained statistically significant (P for all $\leq .01$).

Conclusion: In a community-based cohort, individuals with hepatic steatosis without known liver disease had higher mean serum concentrations of systemic markers of inflammation. Studies are needed to determine whether treatment of hepatic steatosis reduces systemic inflammation.

Introduction

Non-alcoholic fatty liver disease (NAFLD), characterized by hepatic steatosis in the absence of secondary causes, is a common and morbid cause of liver disease worldwide.[1–3] Most people with NAFLD have simple steatosis that does not progress to fibrosis[1], and many consider hepatic steatosis without fibrosis to be an innocuous condition.[4]

Multiple studies have observed associations between NAFLD and cardiovascular disease, including coronary artery calcium,[5,6] microvascular function,[7] and diastolic dysfunction.[6] Systemic inflammation is a major component of cardiovascular disease[8] and is associated with the metabolic syndrome,[9] obesity,[10,11] and diabetes.[12] An increased influx of free fatty acids to the liver may lead to increased transcription of systemic inflammatory mediators, which accelerate cardiovascular disease.[13] Since NAFLD is strongly associated with obesity and insulin resistance, it is important to understand the extent to which NAFLD is associated with systemic inflammation after accounting for potential confounding factors. In several small studies, participants with NAFLD and insulin resistance[14,15] or the metabolic syndrome[16] had higher measures of systemic inflammation compared to those without NAFLD. In hospital-based cohorts, several studies observed an association between those with NAFLD or hepatic fibrosis and measures of systemic inflammation.[17,18,27–29,19–26] Prior studies of the association between NAFLD and inflammatory makers have largely been limited by small sample sizes[16,17,31–33,18,20,24,25,27–30] or measurement of a single or few inflammatory markers[10,14,35,15,19,21,22,24,25,33,34], and have predominantly included those with more advanced NAFLD.[23,26,29] The relationship between hepatic steatosis and systemic inflammation among community-dwellers not selected for liver disease has largely been unexplored.

Thus, we evaluated the association between liver fat as measured on non-contrast enhanced computed tomography and multiple biomarkers of systemic inflammation and oxidative

stress among community-dwelling participants in the Framingham Heart Study (FHS). We hypothesized that liver fat is associated with multiple markers of systemic inflammation even after accounting for obesity and other covariates.

Participants and Methods

Participants (n=3,394) from the FHS second and third generation cohorts who participated in a multi-detector computed tomography substudy were eligible for inclusion.[36] The FHS is a multi-generational community-based epidemiological study of cardiovascular disease in Massachusetts, USA, beginning in 1948, including over 14,000 participants (https://www.framinghamheartstudy.org).[37]

We excluded participants for: inadequate image capture of the liver (n=265); excess alcohol use (>14 drinks/week for women, >21 drinks/week for men, n=484)[2,38]; missing information on alcohol use (n=26); and missing covariates (n=137) (Figure 1). The study protocol was approved by the Institutional Review Board at Boston University Medical Center; all participants provided written informed consent.

Measuring hepatic steatosis

Assessment of hepatic steatosis was standardized relative to a radiographic phantom during eight-slice multi-detector computed tomography (LightSpeed Ultra, General Electric, Milwaukee, WI).[36] Full details of the acquisition protocol have been previously published.[36] In brief, the mean Hounsfield units from three areas of the liver of at least 100 mm^2 (intentionally avoiding blood vessels) was compared to a commercially available calibration control or “phantom,” which was present on all images. Lower values of the Liver:Phantom Ratio (LPR) correspond to more radiographic hepatic steatosis and have previously been shown to be associated with cardiometabolic risk factors.[39] Hepatic steatosis was defined as LPR ≤ 0.33 based on prior studies using the liver to spleen ratio.[36,39] All image processing was overseen and reviewed by a single radiologist (UH) and has previously been demonstrated to have good inter- and intra-rater reliability.[36]

Covariates

Covariates were chosen *a priori* based on their relevance for risk of liver disease or potential influence on a systemic inflammatory state. Covariates included body mass index (BMI, defined by weight (kg) divided by height squared (m^2)), hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or treatment with an antihypertensive medication), cardiovascular disease (history of coronary heart disease, stroke, heart failure, or intermittent claudication adjudicated by review of medical records[40]), or diabetes mellitus (fasting serum glucose ≥ 126 mg/dL or treatment with an anti-hyperglycemic medication). Regular aspirin use was defined as self-reported chronic use of three or more aspirins per week. Lipid-lowering therapy was defined as participant-reported use of medications for “high cholesterol or high triglycerides.” Average alcohol use was participant-reported in drinks per week or drinks per month. Current smoking was defined as one or more cigarette(s) per day over the past year.

Multiple laboratory values also were measured on fasting morning samples, including blood glucose (mg/dL), total cholesterol (mg/dL), high density lipoprotein (mg/dL), cholesterol (mg/dL), triglycerides (mg/dL), and alanine aminotransferase (ALT, U/L). Quantification of visceral adipose tissue (VAT) was done using a semi-automated method previously described which yields excellent inter-reader reproducibility.[41] For a secondary analysis, elevated ALT was defined as an ALT > 19 U/L for women and > 30 U/L for men.

Markers of systemic inflammation and oxidative stress

Inflammatory biomarkers were measured at the same time as covariates and include 14 measurements as listed in Table 1. Technical details for these measurements have been previously described[42,43] and are summarized in the Supplemental information.

Statistical analyses

Values of inflammatory markers were transformed using the natural logarithm prior to all analyses to normalize skewed distributions. Pearson correlation coefficients were calculated to measure the association between LPR and each inflammatory marker. The primary analysis assessed the relationship between LPR and inflammatory markers using multivariable linear regression models of increasing complexity. Model 1 adjusted for age, sex, average alcohol use, regular aspirin use, and smoking status. Model 2 added adjustment for hypertension, total cholesterol:high density lipoprotein ratio, lipid-lowering therapy, triglycerides, diabetes mellitus, and cardiovascular disease. Model 3 additionally adjusted for BMI. We performed multiple sensitivity analyses to exclude participants with diabetes or cardiovascular disease and to additionally adjust for VAT in Model 2. Interactions with sex and obesity (BMI $\geq$ 30 kg/m^2) were also assessed.

We assessed the relationship of the combination of hepatic steatosis and elevated ALT with systemic inflammatory markers. An ANCOVA was used to compare differences in the mean natural log-transformed inflammatory markers between groups after adjusting for the same covariates as Model 1 described above.

An “inflammatory index” was defined as the number of inflammatory markers above the sample median value for each of hs-CRP, ICAM1, IL6, P-selectin, and urinary isoprostanes. These Inflammatory markers were chosen based on the results of the multivariable models.

Statistical tests were conducted at an α =0.05 level of significance. We performed the Benjamini-Hochberg procedure[44] with false discovery rate set at 0.05 or 0.01 to account for multiple testing. All analyses were performed using SAS 9.3.

Results

Summary statistics for demographic characteristics and covariates are presented in Table 2. A total of 2,482 participants with a mean age of 51±11 years were included. Approximately 50% were women. Overall, 17% of participants had hepatic steatosis (as defined by LPR $\leq$ 0.33). Unadjusted inflammatory marker values are presented in Supplement Table 1. We observed statistically significant positive correlations between LPR and multiple inflammatory markers after adjusting for age and sex (Supplement Table 2). We observed a

modest to strong correlation between the inflammatory markers with correlation coefficients ranging from −0.09 to +0.48 (data not shown).

In Model 1, LPR was positively associated with nearly all inflammatory markers measured (with exception of CD40 ligand, Lp-PLA2 mass, MPO, and TNF-α). (Table 3). Multivariable linear regression models with adjustment for additional covariates attenuated the associations between LPR and inflammatory markers. In Model 2, LPR was significantly associated with hs-CRP ($P<0.001$), isoprostanes ($P<0.001$), IL6 ($P<0.001$), ICAM1 ($P<0.001$), and P-selectin ($P=0.01$). In Model 3, (with additional adjustment for BMI) these associations were further attenuated but remained statistically significant (P for all ≤ 0.05). Among these markers, a significant interaction with sex was present for only isoprostanes. When results from men and women were analyzed separately, there was no change in the direction of association, but the magnitude was greater for women. Similarly, a significant interaction with obesity was found for only CRP and IL6. The magnitude of association was greater for CRP and IL6 among obese (BMI ≥ 30) participants. (Supplement Table 3).

Using the Benjamini-Hochberg procedure, we evaluated for multiple testing at a false discovery rate threshold of 0.05 and 0.01. Using either threshold, all of the unadjusted p-values that were statistically significant in Models 1–3 remained statistically significant after adjustment for multiple testing.

We conducted a sensitivity analysis to adjust for radiographic VAT in place of BMI in Model 3. Results were consistent with the primary analysis (Supplement Table 4). We also repeated Model 3 after excluding participants with diabetes or cardiovascular disease (287 excluded). Results were consistent in strength and direction of association (Supplement Table 5).

We performed an analysis to compare participants with hepatic steatosis with elevated ALT (n=245) to participants with hepatic steatosis without elevated ALT (n=180). After adjustment for the variables included in Model 1, we found statistically significantly greater ICAM1 ($P<0.0001$), LP-PLA2 activity ($P=0.0002$), P-selectin ($P=0.0001$), and TNFR2 ($P=0.0001$) among those with elevated ALT (Supplement Table 6).

An inflammatory index was calculated for participants with measurements for hs-CRP, ICAM1, IL6, P-selectin, and urinary isoprostanes (n=2,281,). The prevalence of hepatic steatosis was greater among participants with a higher inflammatory index (Figure 2). For participants with an inflammatory index of 0, the prevalence of hepatic steatosis was 3%, whereas for participants with an inflammatory index of 5 the prevalence of hepatic steatosis was 44%.

Discussion

In a cohort of community-dwelling participants without pre-selection for the presence of liver disease, we observed a statistically significant association between liver fat and markers of systemic inflammation and oxidative stress. After accounting for multiple covariates, including BMI, liver fat was positively associated with hs-CRP, isoprostanes, IL6, ICAM1, and P-selectin. Of these, ICAM1 and P-selectin were significantly greater in participants with hepatic steatosis and elevated ALT compared to those with hepatic steatosis without

elevated ALT. Among those with greater inflammation, we observed a stepwise greater prevalence of hepatic steatosis.

The mechanisms linking hepatic steatosis to systemic inflammation independent of other cardiometabolic risk factors remain uncertain. In a prior FHS analysis, VAT, not subcutaneous fat, was associated with hs-CRP, IL6, and urinary isoprostanes, after accounting for other measures of obesity.[42] Our findings expand on this prior study to suggest that hepatic fat remains associated with multiple inflammatory markers after accounting for general adiposity (BMI) or central adiposity (VAT). Prior studies have shown that exposure of hepatocytes to fatty-acids induces expression of TNF-α,[45,46] IL6,[21,46] ICAM1,[47] and isoprostanes,[48] possibly mediated via nuclear factor-κB.[49] Chronic hepatic activation of the nuclear factor-κB pathway promotes IL6-mediated insulin resistance[50] and inhibition of TNF-α attenuates hepatic fatty acid oxidation and insulin resistance driven by Kupffer cell activation.[51]

The associations we observed reflect relationships between certain inflammatory biomarkers and atherosclerotic risk, which is more common among individuals with NAFLD.[52] P-selectin is associated with increased risk of coronary heart disease[53] and metabolic syndrome.[54] IL6[55] and ICAM1[56] also appear to have significant roles in atherogenesis. Elevated isoprostanes are associated with increased carotid artery stiffness,[57] and hs-CRP is associated with incident cardiovascular events.[8]

Prior studies have shown a similar association between hepatic steatosis and systemic inflammation.[10,14,23–31,33,15,35,58,16–22] In smaller studies of specific populations, radiographic hepatic steatosis is positively associated with hs-CRP, IL6, and ICAM1, consistent with our results.[30,58] Small studies also show greater hs-CRP among those with hepatic steatosis and nonalcoholic steatohepatitis (NASH).[17–19] IL6 is associated with the degree of hepatic inflammation and fibrosis among smaller cohorts of subjects with NAFLD.[18,21] Unfortunately, the use of a subjects selected for NAFLD or NASH may generate referral bias which limits the generalizability of most prior reports. Our study confirms these results, and because we studied a cohort without pre-selection for liver disease, our findings show that even asymptomatic hepatic steatosis could have consequences for the overall health of all patients. We hypothesize that greater levels of systemic inflammation may be associated with more rapid liver disease progression and a higher incidence of liver-related clinical events; longitudinal studies are warranted to test these hypotheses. Similarly, interventional studies should be pursued to determine if efforts to ameliorate NAFLD (e.g. weight loss or exercise) reduce systemic inflammatory burden. These markers may be useful measures of the effectiveness of interventions to reduce hepatic steatosis and as has been shown in rodent models, may serve as potential targets for treatments to reduce the systemic impact of metabolic syndrome, though future study is needed.[50,51]

The major strength of our study was the enrollment of a moderately large number of participants from within a well-characterized cohort without selection based on a known diagnosis of liver disease. Nonetheless, limitations should be considered. Our sample was largely middle-aged and older adults of European descent, which may limit applicability to other populations. While computed tomography assessment of hepatic steatosis is an

accurate modality in common clinical use, results may differ from histological findings. Importantly, computed tomography does not permit differentiation of simple steatosis from NASH. Hence, in our study, we were unable to examine the systemic inflammatory burden among individuals with simple steatosis compared to NASH or NASH with fibrosis. Prior research estimates the prevalence of advanced fibrosis among those with NAFLD to be near 10%[59], thus we expect the overall prevalence of advanced fibrosis to be low in our sample. Additional studies in participants with more advanced disease are needed to determine the systemic inflammatory burden across the spectrum of NAFLD. The biomarkers studied have a role in other non-cardiac disease states, but it is possible that others not included here may have important associations with NAFLD. The sample size for each biomarker was slightly different and CD40, fibrinogen, MPO, and TNF-α had less available data and had less statistical power for identifying associations. Our study was cross-sectional and observational, and we cannot exclude residual confounding nor establish a causal relation between liver fat and systemic inflammation.

In conclusion, we found that liver fat is positively associated with markers of systemic inflammation among participants without pre-selection for clinical liver disease. This suggests that even without a known diagnosis of NAFLD or NASH, hepatic steatosis may have important systemic consequences. Further studies to characterize these relationships and the underlying causal mechanisms are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ALT	Alanine aminotransferase
BMI	Body mass index
CD40	Cluster of differentiation 40
FHS	Framingham Heart Study
hs-CRP	High-sensitivity C-reactive protein
ICAM1	Intercellular adhesion molecule 1

IL6	Interleukin 6
LP-PLA2	Lipoprotein-phospholipase A2
LPR	Liver:Phantom ratio
MCP-1	Monocyte chemoattractant protein 1
MPO	Myeloperoxidase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
TNF	Tumor necrosis factor
TNFR2	Tumor necrosis factor receptor 2
VAT	Visceral adipose tissue

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What you need to know:

Background:

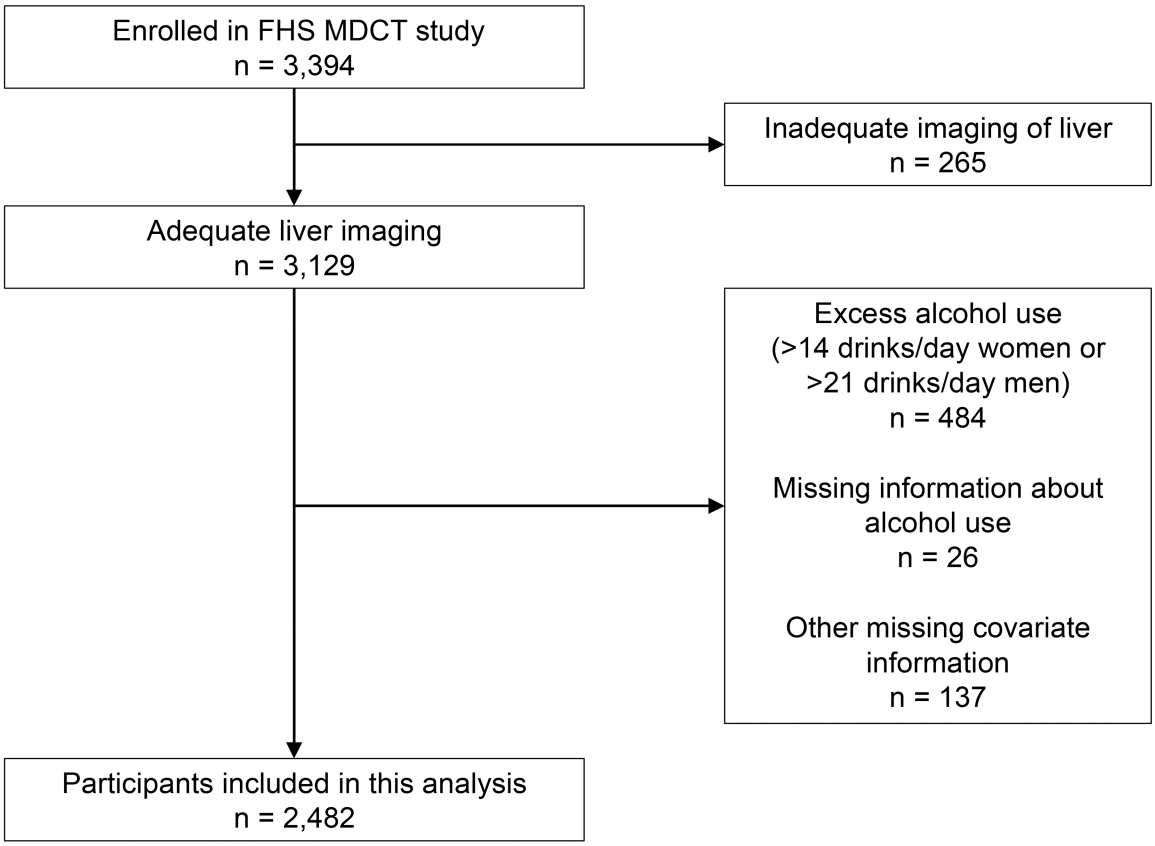
Patients with clinically apparent non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) have higher levels of systemic inflammation. It is unknown whether this is also true in the community setting among patients without pre-selection for liver disease.

Findings:

Among 2,482 participants without pre-selection for liver disease, we observed a positive association between more liver fat (measures by computed tomography) and multiple markers of systemic inflammation, even after accounting for covariates including body mass index.

Implications for patient care:

Individuals with NAFLD, even without clinically apparent liver disease, may have increased systemic inflammation, not explained by common comorbidities. This may increase risk of cardiovascular or other diseases.



FHS (Framingham Heart study)
MDCT (Multi-Detector Computed Tomography)

Figure 1:
Study sample and participant exclusions

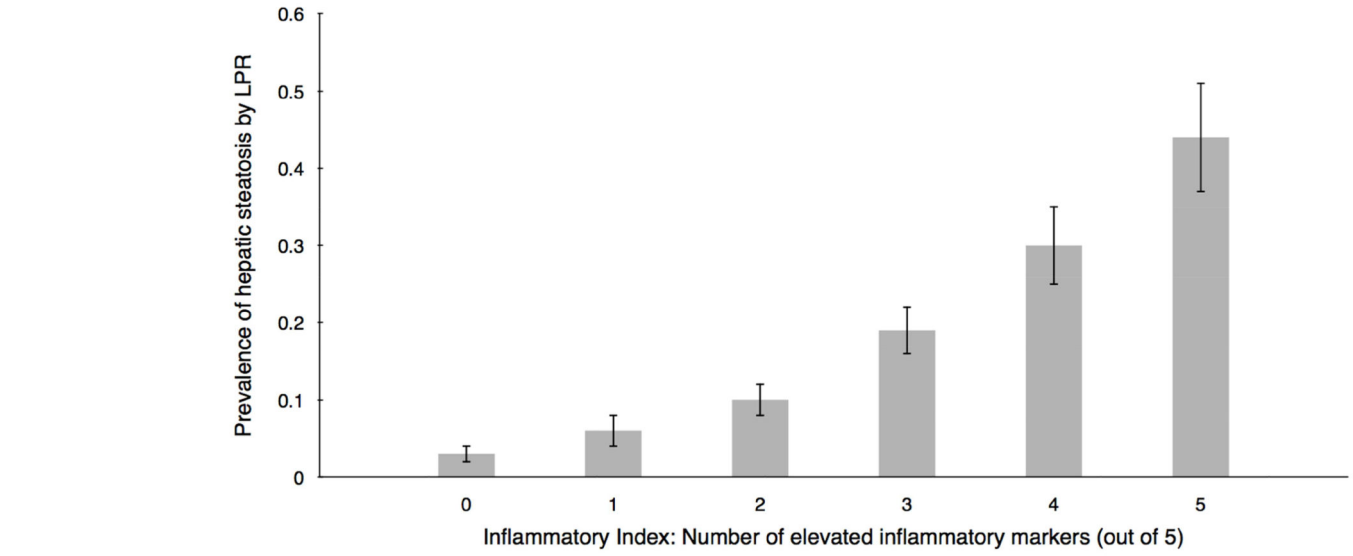


Figure 2:
Prevalence of hepatic steatosis is greater among those with higher inflammatory index

Table 1:

Markers of inflammation and oxidative stress measured

Marker of inflammation	Biological relevance
CD40 ligand (ng/mL)	Marker and mediator of inflammation in atherosclerosis[60]
Fibrinogen (mg/dL)	Marker of thrombosis and inflammation[8]
High-sensitivity C-reactive protein (hs-CRP, mg/L)	Marker of systemic inflammation, strong association with cardiovascular disease[8]
Intercellular adhesion molecule 1 (ICAM1, ng/mL)	Marker associated with progressive atherosclerosis[61]
Interleukin 6 (IL6, pg/mL)	Pro-inflammatory marker[62]
Lipoprotein-associated phospholipase A2 activity (Lp-PLA2 activity, nmol/mL/min)	Low density lipoprotein associated with atherosclerosis[63]
Lipoprotein-associated phospholipase A2 mass (Lp-PLA2 mass, ng/mL)	Low density lipoprotein associated with atherosclerosis[63]
Monocyte chemoattractant protein 1 (MCP1, pg/mL)	Chemokine associated with endothelial damage and atherosclerosis[64]
Myeloperoxidase (MPO, ng/mL)	Marker associated with atherosclerosis and cardiovascular disease[65]
Osteoprotegerin (pmol/L)	Marker of bone metabolism and vascular inflammation[66]
P-selectin (ng/mL)	Marker associated with inflammatory cell adhesion and atherosclerosis[53]
Tumor necrosis factor receptor (TNF-α, pg/mL)	Regulator of the inflammatory response[67]
Tumor necrosis factor receptor 2 (TNFR2, pg/mL)	Marker of inflammation associated with atherosclerosis[68]
Urinary isoprostanes (pg/mL) Marker of oxidative stress	Marker of oxidative stress[69]

Table 2:

Study sample characteristics by hepatic steatosis status

	Hepatic steatosis (n = 425)	No hepatic steatosis (n = 2057)	Overall (n = 2482)
Demographic characteristics and covariates			
Age (years)	52±11	51±10	51±11
Sex (women)	192 (45)	1075 (52)	1267 (51)
Body Mass Index (BMI, kg/m^2)	31±6	27±5	28±5
Current smoking	47 (11)	233 (11)	280 (11)
Former smoking	183 (43)	773 (38)	956 (39)
Cardiovascular disease	43 (10)	126 (6)	169 (7)
Regular aspirin use	107 (25)	379 (18)	486 (20)
Alcoholic drinks per week	3.1±3.9	3.0±3.4	3.0±3.5
Diabetes	61 (14)	101 (5)	162 (7)
Fasting glucose (mg/dL)	108±33	97±19	99±22
Hypertension	192/424 (45)	486/2055 (24)	678/2479 (27)
Lipid lowering therapy	84 (20)	249 (12)	333 (13)
Total cholesterol (mg/dl)	197±36	194±35	195±35
High-density lipoprotein (HDL) cholesterol (mg/dl)	45±14	54±16	52±16
Triglycerides (mg/dl)	180±115	115±75	126±87
Alanine aminotransferase (ALT, U/L)	34.1±25.8	23.1±15.8	25.0±18.4
Visceral adipose tissue (VAT, cm^3)	2626±1049	1565±912	1747±1019

Mean±standard deviation for continuous variables or n (%) for categorical variables

Hepatic steatosis defined by Liver:Phantom Ratio ≤ 0.33

See *Methods* for covariate definitions

Table 3:

Multivariable linear regression models of the relationship between liver fat (by Liver:Phantom Ratio) and markers of inflammation and oxidative stress

Markers of inflammation or oxidative stress	Model 1		Model 2		Model 3	
	β (95% CI)	***P*-value**	**β (95% CI)**	***P*-value**	**β (95% CI)**	***P*-value**
Cluster of differentiation 40 (CD40) ligand	0.23 (−0.52, 0.99)	0.55	0.38 (−0.41, 1.17)	0.35	0.47 (−0.34, 1.27)	0.26
Fibrinogen	**0.18 (0.08, 0.29)**	**<0.001**	0.10 (−0.01, 0.21)	0.07	0.02 (−0.08, 0.13)	0.67
High-sensitivity C-reactive protein (hs-CRP)	**3.05 (2.61, 3.48)**	**<0.001**	**2.20 (1.75, 2.64)**	**<0.001**	**1.23 (0.81, 1.65)**	**<0.001**
Intercellular adhesion molecule 1 (ICAM1)	**0.44 (0.34, 0.53)**	**<0.001**	**0.34 (0.24, 0.43)**	**<0.001**	**0.30 (0.20, 0.40)**	**<0.001**
Interleukin 6 (IL6)	**1.14 (0.88, 1.40)**	**<0.001**	**0.84 (0.57, 1.11)**	**<0.001**	**0.50 (0.23, 0.77)**	**<0.001**
Lipoprotein-phospholipase A2 (Lp-PLA2) activity	**0.09 (0.01, 0.17)**	**0.03**	−0.02 (−0.09, 0.05)	0.62	0.02 (−0.05, 0.09)	0.62
Lipoprotein-phospholipase A2 (Lp-PLA2) mass	−0.06 (−0.16, 0.04)	0.27	−0.08 (−0.19, 0.02)	0.11	−0.09 (−0.19, 0.02)	0.11
Monocyte chemoattractant protein 1 (MCP1)	**0.16 (0.03, 0.30)**	**0.02**	0.10 (−0.04, 0.24)	0.16	0.07 (−0.08, 0.22)	0.34
Myeloperoxidase (MPO)	−0.03 (−0.38, 0.32)	0.86	0.00 (−0.37, 0.37)	0.99	−0.13 (−0.51, 0.25)	0.50
Osteoprotegerin	**0.14 (0.02, 0.27)**	**0.03**	0.11 (−0.02, 0.24)	0.10	0.13 (−0.01, 0.26)	0.07
P-selectin	**0.48 (0.32, 0.63)**	**<0.001**	**0.25 (0.10, 0.41)**	**0.002**	**0.20 (0.04, 0.36)**	**0.01**
TNF receptor-2 (TNFR2)	**0.21 (0.10, 0.32)**	**<0.001**	0.11 (−0.01, 0.22)	0.08	0.02 (−0.10, 0.14)	0.76
TNF-alpha (TNF-α)	0.09 (−0.25, 0.43)	0.59	−0.09 (−0.45, 0.26)	0.6	−0.21 (−0.57, 0.15)	0.25
Urinary isoprostanes	**1.40 (0.99, 1.81)**	**<0.001**	**1.15 (0.72, 1.58)**	**<0.001**	**0.87 (0.43, 1.31)**	**<0.001**

Estimates in table give change in $\log_e$ biomarker for a 1 standard deviation increase in liver fat (measured via the liver phantom ratio) with (95% CI) and p-value

Model 1 adjusts for age, sex, smoking status, alcohol consumption, and regular aspirin use.

Model 2 additionally adjusts Model 1 for hypertension, lipid treatment, total/high density lipoprotein cholesterol ratio, triglycerides, diabetes, and prevalent cardiovascular disease.

Model 3 additionally adjusts Model 2 for body mass index

See *Methods* for covariate definitions